A simplified interface for handling microtitre plate assays with the Hamilton Microlab STAR

Neville Ng[1,2], Reece Gately[1], Lezanne Ooi[1,2,*]

Manuscript ID: JAB-2020-6-2/R1

DOI: 10.17145/jab.20.xxx

To be published in: *Journal of Applied Bioanalysis (ISSN 2405-710X)*

Received Date: 22 June 2020

Revised date: 28 July 2020

Accepted Date: 02 August 2020

Please cite this article as: Ng N, Gately R, Ooi L. A simplified interface for handling microtitre plate assays with the Hamilton Microlab STAR. J Appl Bioanal (2020), forthcoming.

A simplified interface for handling microtitre plate assays with the Hamilton Microlab STAR

Neville Ng[1,2], Reece Gately[1], Lezanne Ooi[1,2,*]

[1]Illawarra Health and Medical Research Institute, Wollongong, New South Wales, Australia

[2]School of Chemistry and Molecular Bioscience and Molecular Horizons, University of Wollongong, New South Wales, Australia

*Correspondence should be addressed to:

IHMR, University of ,Wollongong, Building 32, Wollongong, 2522 Australia.

e-mail:lezanne@uow.edu.au

Abstract

Automated liquid handling stations such as the Hamilton Microlab range can be implemented to greatly enhance throughput of cell-based and cell-free assays. To facilitate utilisation past the comprehensive programming interface of the Hamilton Method Editor this paper presents a user interface (UI) that runs within Hamilton Venus and allows for a user to control and store programs for plate-to-plate transfers and serial dilutions in 96 well plate format. The interface allows rapid control of aspiration and dispensing height, trituration, control of tip rack selection, and includes a tip washing program that can reduce the number of disposable tips utilised. The simple program interface allows the Hamilton Microlab to be used as a readily deployable microtitre plate handler, particularly for applications such as aliquoting cells for seeding, diluting a number of plates in medium, or transferring drug dilution arrays to multiple plates. This paper also discusses various optimisations to increase accuracy and rate of low volume liquid transfer. While complex liquid handling tasks such as high-throughput drug screening requires established core facilities, standalone liquid handlers with simplified interfaces can be utilised for smaller-scale research applications and educational purposes.

Introduction

Automated liquid handling can greatly accelerate the progress and efficiency of biochemical or cell-based assays. For small to medium throughput assays, such as investigating a several thousand compound drug library in a single condition, or dose response assays with multiple conditions, mid-range robotic liquid handlers offer a convenient means of controlling dozens of microtitre plates. While automated high throughput drug screening typically involves equipment

and set-ups for specific routines, medium throughput automated liquid handling is comparably affordable and circumvents the high cost of purchasing, maintaining or lease of high-throughput robotics, coupled with more adaptability. The advantage of the 96 well plate format as opposed to miniaturised variants (384 and 1536 well plates) particularly for cell-based assays is pipetting accuracy with medium rather than low volume handling and troubleshooting.

Mid-range robotic liquid handlers such as the Hamilton Microlab are typically configured for genomics, biochemistry or cell culture handling setups with a versatile range of specialised labware. For purposes described, the Hamilton Microlab supports a 96 CO-RE head as well as an 8-channel array with proprietary tip pickup and ejection. While the Hamilton Microlab offers robust liquid handling with superior consistency when compared to manual pipetting, its native programming interface is intrinsically complex. To reduce the level of configuration and learning curve, a simplified user interface within the Hamilton Method Editor has been designed, the Hamilton Microlab Handler 96 (HMH96), that simplifies the number of steps and considerations involved in plate-to-plate transfer, and includes aliquoting, serial dilution, spotting, and tip washing for 96 well plates. A significant drawback of proprietary robotic liquid handling stations is that tip selection is often limited to proprietary sources which with features such as reinforced strength or conductivity but may be more expensive than typical tips. To address this HMH96 includes tip wash functions.

The Hamilton Microlab consists of highly customisable liquid handling and vessel handling devices, with plates or tips are mounted in carriages that are inserted and drawn forwards or backwards. For HMH96 microtitre plate handling, the instrumentation setup requires a CO-RE 96 probe head, and for serial dilutions, an 8-channel array of 1000 µL channel pipettes. This setup relies on low and medium volume pipetting (2-200 µL) require 50 µL and 300 µL tip

racks. HMH96 is a user interface dialog designed within Hamilton Method Editor. iSWAP control is included for lid handling however is not essential for function. HMH96 simplifies liquid handling to 4 main classes that can be calibrated via the default Hamilton CO-RE Liquid Editor. Setup for deck layout is not altered and relies on configuration via the default Hamilton Method Editor. After deck layout and liquid class configuration, the user can easily control the 96 probe head to aspirate from a source and dispense to a number of elected plates by position name ('sequence') with aspiration and dispense level, trituration during aspiration, and jet or surface empty mode upon dispense. The serial dilution program is similarly customisable for column to column dilutions. Before and after usage, the tip wash program can be used to rinse an array of pipette tips in an elected vessel before emptying.

HMH96 functions as a dialog within the Venus program and stores user settings in the same folder as the method directory, in a text file which contains a string of semi-column delimited variables in a continuous string per line written and parsed by Venus software.

In this article, considerations of low volume pipetting are also discussed, including disabling specific features such as air transport volume or liquid level detection to increase rate of liquid transfer. The same concepts demonstrated by this liquid handling interface and liquid handling considerations for accuracy, washing, and sterility are applicable to other programmable liquid handlers.

Experimental

Instrumentation. HMH96 requires a 96 CO-RE head with or without Total Aspiration and Dispense Monitoring (TADM), 8-channel CO-RE 1000 µL channel head and large iSWAP

gripper. HMH96 was designed within Hamilton Venus 2 for a Hamilton Microlab STAR, and is likely compatible with later versions of Venus and other Microlab platforms.

Deck layout. This interface requires the layout to be correctly configured in the default Hamilton Method Editor in a .lay file, and can be adjusted to suit needs. Care must be taken to select the correct Labware type, correct xyz positioning and particularly labware base height. The default setup deck layout of HMH96 involves 3 carriages of 5 plates, 1 carriage of 5 x 50 µL tip racks and 1 carriage of 5 x 300 µL tip racks. The SBS 96 well flat bottom, U-bottom, or V-bottom microtitre plates, or deep wells, all share a similar base height, but these should be checked at each position to avoid unexpected aspiration/dispense heights. Care should be taken to ensure all tips contact the base of plate to ensure accuracy of spotting functions. The HMH96 method file requires each vessel (assigned by sequence) to be added to a PlateArray, and requires “Aux” to be an empty position for lid handling.

Liquid classes. The interface relies on importing of liquid classes used by the 96 head pipettor are jet empty and surface empty for 300 µL tips and 50 µL tips, packaged in an .mdb file. HMH96 is primarily intended for handling aqueous solutions or low volumes of DMSO, which are relatively similar in handling by liquid class.

Interface. The method file is loaded in Hamilton Run Control and started; after initialisation, the HMH96 dialog will appear. Programs can be added, deleted, moved, and saved. To add a new program, “+”, “-“. “<” or “>” changes currently selected program accordingly, “<<” and “>>” changes the currently selected program to start or end. “S<” or “S>” switches current program

with previous or next program. Positions designated by sequence names appear in the right hand-column. An error will trigger if an invalid position is written in position text boxes.

Depending on program, aspiration volume, height, retraction distance from aspiration level, and number of triturations during aspiration can be selected. For optimal intrawell accuracy, all liquid handling is performed by surface empty methods unless “Jet” is selected. The dispense section allows selection of dispense volume, dispense height, and retraction height after dispense.

Common to all programs is the tip control, available in the Tips section. The left dialog indicates which tip rack to use, and, more relevant to wash and purge steps, how many racks to process. “Lid” designates removal of lid to “Aux”, which is an empty platform for the iSWAP to remove a lid to before processing, and return to plate after aspiration or dispense.

1.2 Plate-to-plate liquid transfer

The Aliquot program is the routine plate-to-plate transfer method (Figure 1). “Pre-rinse” indicates a vessel to dip tips into before beginning aspiration, and the adjacent text box is the height to lower tips to. “Return excess” indicates whether to dispense remaining volume to source. “Side touch” controls whether to touch the upper right-hand side level of the labware-configured side touch height after dispensing.

Disabling liquid level detection reduces chance of sporadic error while preserving practical intrawell error. Without liquid level detection, dispensing height must be selected accurately; for example, an aqueous solution at 100 µL in a standard ~0.32 cm^2 surface area well 96 well plate should be dispensed at 0.51 mm above base. Default liquid classes used by HMH96 disable air transport volume and eliminate need for retraction height between transfers, which can increase a

considerable amount of time with default settings. Including a trituration cycle during aspiration may improve consistency with cell or chemical suspensions. As using an iSWAP for lid handling greatly increases the duration of aspiration/dispense cycles and requires a placeholder plate to place lid on, we recommend manual lid removal for most practical liquid handling applications.

1.3 Low volume plate-to-plate liquid transfer

The Aliquot (Figure 1) and Spot methods (Figure 2) allow for accurate low volume pipetting to empty plate. Accuracy is highest when dispensing into a volume of liquid with excess aspiration volume, however in some cases this may not be easily performed, for example preparation of drug library plates. For this purpose it is more rapid and accurate to use the Aliquot program that uses the 96 head probe in a V-bottom 96 well plate, ensuring volumes are above 5 µL and all pipette tips are within closest proximity of the base of the plate and that tips are fully submerged throughout the dispensing process. However, with low volumes slight angles may prohibit this, and require V-bottom plates. The Spot method employs the 8-channel probe in bottom-touch mode to ensure maximal practical contact with plates and can be employed with 96 well flat bottom plates, at a considerable loss of speed.

Serial dilutions

The Dilute method sequentially aspirates a specified volume from a specified column of wells, dispense to a right adjacent column and mix, until the end column is reached, allowing for serial dilution of multiple plates designated by sequence position (Figure 3). In Aspirate settings,

aspiration level, distance to retract after aspiration, and triturations to mix can be adjusted. In Dispense settings, dispense height and retraction distance can be adjusted. In the Dilute Array dialog, the first column and last column of serial dilution per microplate are selected.

1.4 Tip Wash and Purge

The Tip Wash routine (Figure 4) requires a wash vessel to be selected and a purge vessel (an empty 96 well plate). The interface allows for the position of tip (pos) and number of racks to wash (#). The program involves 3 steps: jet empty in waste (by default Waste96), rinse in container first elected container, and jet empty and side touch in second elected container. There is no simple method of purging tips with built in Hamilton Method Editor functions; the Tip Purge program relies on picking up and replacing tips to induce expulsion of residual volume. This increases rate of drying of the tips after a Tip Wash program. Selecting an adequate trituration number and using multiple wash vessels with adequate volume is necessary for effective washing.

Discussion

Versatile platforms such as the Hamilton Microlab can be difficult to share between users and risk of collision and equipment damage is imminent if deck layouts do not match the configuration present. In shared instrumentation setups, it is recommended to utilise the Hamilton Microlab on a project-specific basis or agreement on a specific layout and purpose.

Pipetting Accuracy

The accuracy of the Hamilton Microlab CORE96 head is evident with intra-well CV that are comparable to commercial information. While conductive and pressure based liquid level detection offer advantages of error detection during pipetting error and automatic detection of liquid level, this has been found to trigger spurious errors and is not necessary to maintain practical levels of accuracy. When applied with HMH96, the expected CV with 50 µL tips in surface empty mode to dispense 5 µL, or 300 µL tips to dispense 100 µL in surface empty or jet empty modes is approximately ~2%, which is comparable to manual multi-channel pipetting. For volumes at approximately 2 µL, a CV ~5% could be achieved with a pre-existing volume (Figure 5). As a precautionary stage it is ideal to prime tips by aspiration and dispensing to the same vessel as the first stage of each sequence. We found that for low volume pipetting, a 2 µL excess volume may provide a minor increase in consistency. We also found that removing air transport volume and retraction, which can add several seconds to each aspiration-dispense cycle, does not reduce accuracy of pipetting, and with no visible leakage of liquid when handling aqueous or DMSO solvents. To reduce chance of dislodging cells in cell-based assays, the dispense rate of the surface empty method was also decreased; it is not recommended to employ jet empty methods for seeding or adding treatment or fixatives to cell layers.

Spotting has been found to be challenging with solvents such as DMSO on the flat base of the 96 well plates. As low as 5 µL can be successfully transferred in V-bottom plates with 8 channel or 96 CORE probe, however volumes lower than this have been found to have poor accuracy. The accuracy of the Spot program can be variable and in flat bottom plates it relies on surface tension to keep tips fully submerged, so accuracy decreases considerably if using DMSO. Thus, the Spot program should be used only for low volume dispensing of aqueous solutions greater than 5 µL

when using flat-bottom 96 well microtitre plates. Attempting to Spot volumes less than 2 µL is not feasible with this program due to the level of inaccuracy measured.

Tip reuse and washing

The Tip Wash routine has been found effective with at least 2 triturations for either standard 300 µL or small 50 µL tips for removing aqueous solutions in 100 mL of H_2O (Figure 7). Experiment-specific optimisations for effective tip washing are required depending on solvent composition type. We have reused tips utilised for cell-based assays and working solution small molecule addition (µM range), without compromising results, however do not recommend reuse and washing or tips utilised for high concentration substances (*e.g.* small molecule handling in high mM range) since effective washing is limited to reservoir volume from each wash.

Live-cell applications

For mammalian cell culture applications, although a HEPA filter module is essential for antibiotic-free handling, we have found low contamination rate is feasible with a standard enclosure and typical concentrations of antibiotics (100 U/mL penicillin/streptomycin) with adherence to basic aseptic principles, including ethanol sterilisation of gloves and vessel edges (Figure 6). Inclusion of antimycotics may allow for continuous cell culture applications however this is not recommended due to long term impact on mammalian cells. For enclosure-only Hamilton Microlab setups it is likely that cell-based assay experiments are limited term end-point assays (< 7 days), which can be addressed by installation of a HEPA filter. It is unlikely that the basic enclosure will be suitable for antibiotic-free experiments including microbiological applications without a HEPA filter due to internal airflow during mechanical operation.

Although robotic liquid handlers enable scalability of microtitre cell-based assays, typical considerations must be addressed to avoid generation of artefactual data, including incubation of cells ambient temperature for < 0.5 h to avoid edge effect aggregation (1), use of a close humidifier system to prevent evaporation over extended incubation periods, particularly when including usage of edge wells (2), and use of pre-heated metallic blocks that allow for direct contact to base of microtitre plates (3). Thermal heating gradients are not limited to cell-based assays but are also known to affect reactions such as ELISA immunolabelling procedures (4). We have found that preheating solution in absence of a heating block for incubation is insufficient to ameliorate thermal gradient artefacts, and where metallic heating blocks are not practical for large scale assays, it may be more ideal to perform incubations at ambient temperature instead (Figure 8). This consideration is not limited to metabolic probe incubation; issues have been encountered with loading of intracellular probes such as tetramethylrhodamine for mitochondrial membrane potential assays, or 2',7'-dichlorodihydrofluorescein diacetate for reactive oxygen species detection.

Conclusion

Robotic liquid handlers controlled by simple user interfaces such as HMH96 can be utilised to seed and maintain live cells for cell-based assays, conduct metabolic probe assays, and fix, wash and stain cells with immunolabelling or total protein dyes. Low volume pipetting can also be incorporated to facilitate drug library aliquots and RT-qPCR applications. The aspiration and dispense cycles are considerably rapid and accurate compared to manual handling (e.g. ~5s per plate transfer with low volumes). The fundamental liquid handling principles, considerations for

accurate pipetting, washing, sterility and limitations explored in this article, as well as the general format of the HMH96 user interface are universally applicable to other robotic liquid handling concepts. The challenges of avoiding artefactual data caused by uneven seeding, thermal gradients, microtitre cell-based and cell-free assays. Although the automation of tasks such as high throughput drug screening is best delegated to established core facilities with well-integrated liquid handling, analysis and incubator systems, simplified graphical interfaces allow for utilisation of stand-alone liquid handlers for smaller scale applications in research or educational purposes.

Acknowledgements

We are appreciative to Dr Amee George ANU Centre for Therapeutic Discovery for high throughput drug screening technical advice.

1. Lundholt BK, Scudder KM, Pagliaro LJJobs. A simple technique for reducing edge effect in cell-based assays. 2003;8(5):566-70.

2. Walzl A, Kramer N, Mazza G, Rosner M, Falkenhagen D, Hengstschläger M, et al. A simple and cost efficient method to avoid unequal evaporation in cellular screening assays, which restores cellular metabolic activity. J International Journal of Applied Science Technology. 2012;2(6).

3. Shellman YG, Ribble D, Yi M, Pacheco T, Hensley M, Finch D, et al. Fast response temperature measurement and highly reproducible heating methods for 96-well plates. J Biotechniques. 2004;36(6):968-76.

4. Mushens R, Scott M. A fast and efficient method for quantification of monoclonal antibodies in an ELISA using a novel incubation system. J Immunol Methods. 1990;131(1):83-9.

Figures

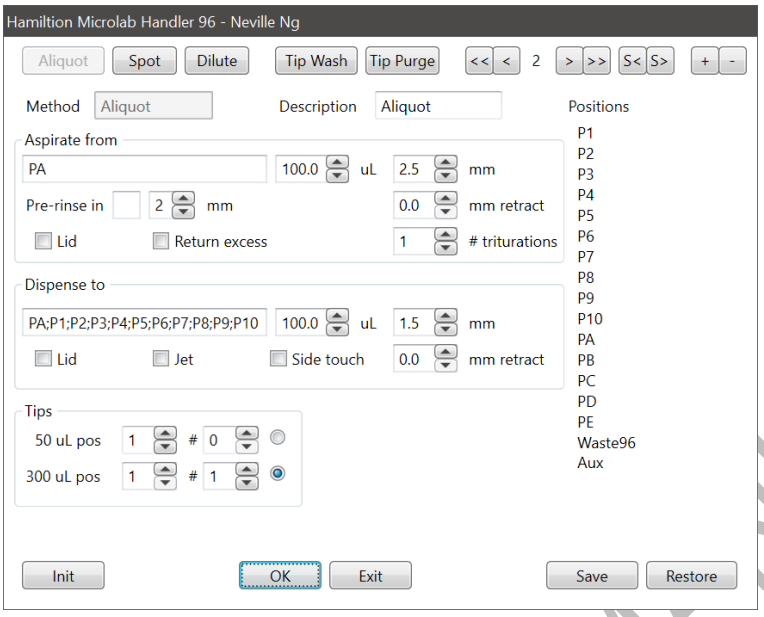

Figure 1. Aliquot program interface

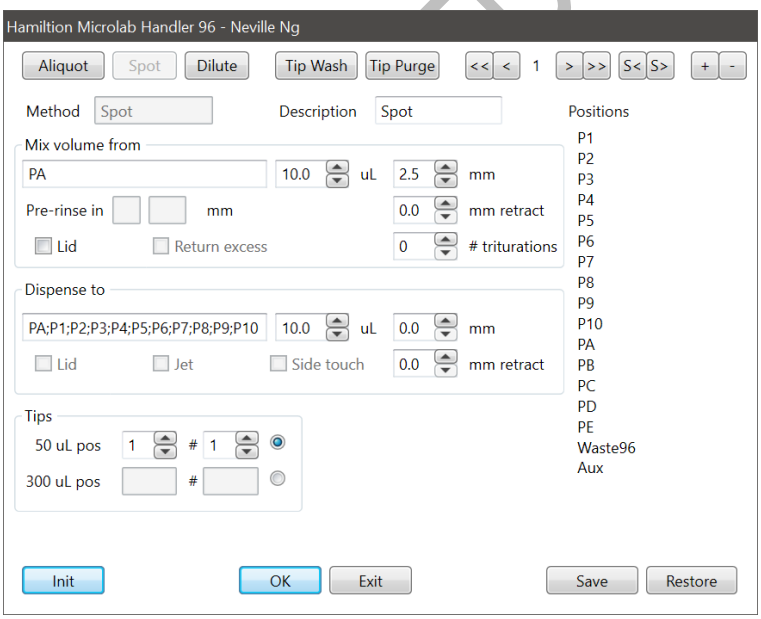

Figure 2. Spot program interface

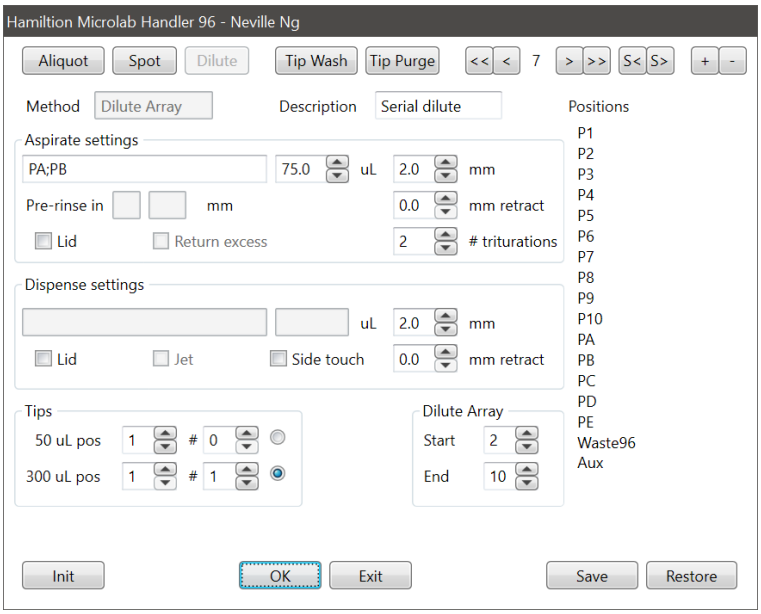

Figure 3. Serial dilution program interface

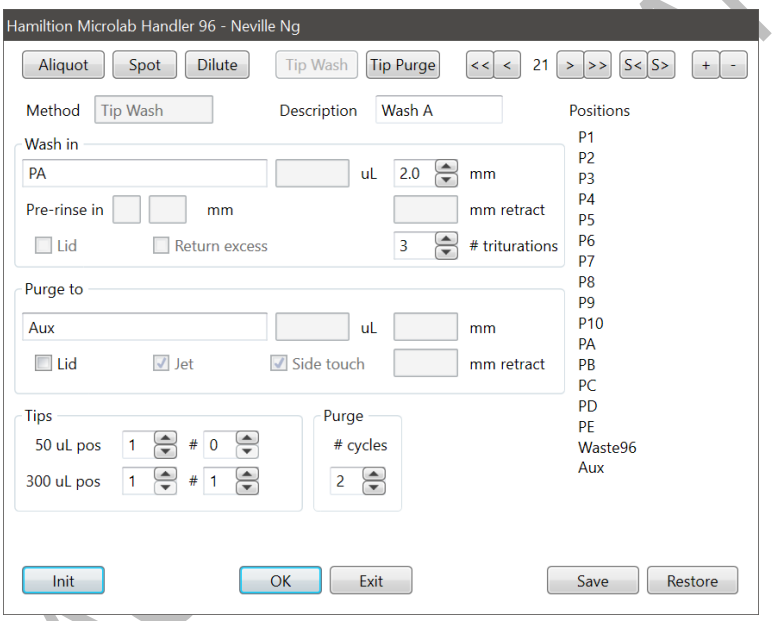

Figure 4. Tip wash program interface

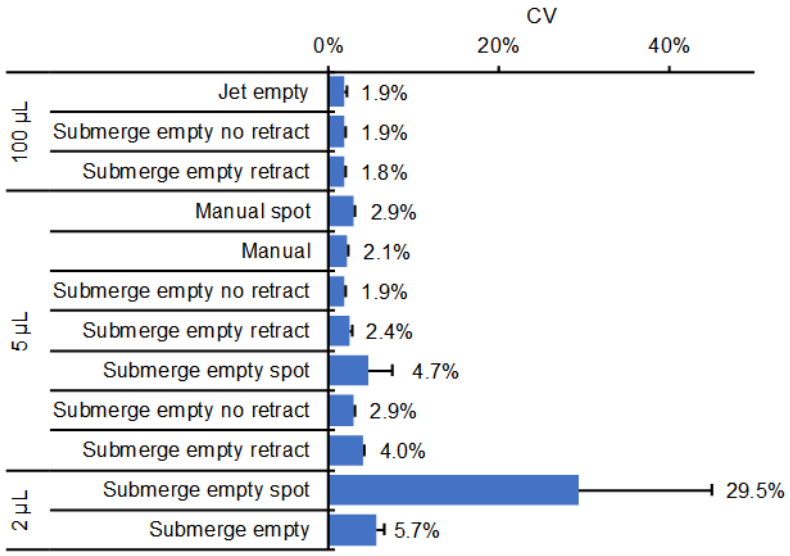

Figure 5. Intraplate CV measured with sulforhodamine B (0.004% w/v) by fluorescent plate spectroscopy (n = 2, error presented as standard deviation).

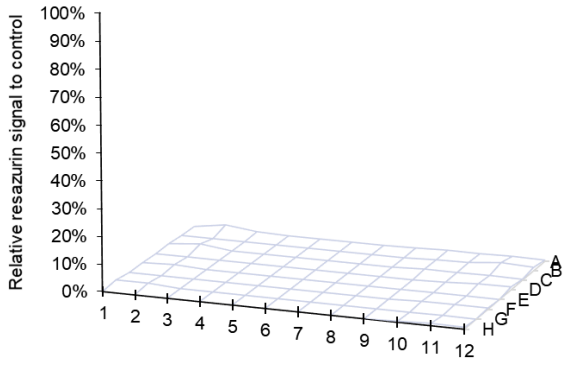

Figure 6. Assessment of microbial growth in DMEM/F12 medium with 100 U/mL penicillin/streptomycin following overnight incubation relative to signal from confluent human fibroblasts (n = 2).

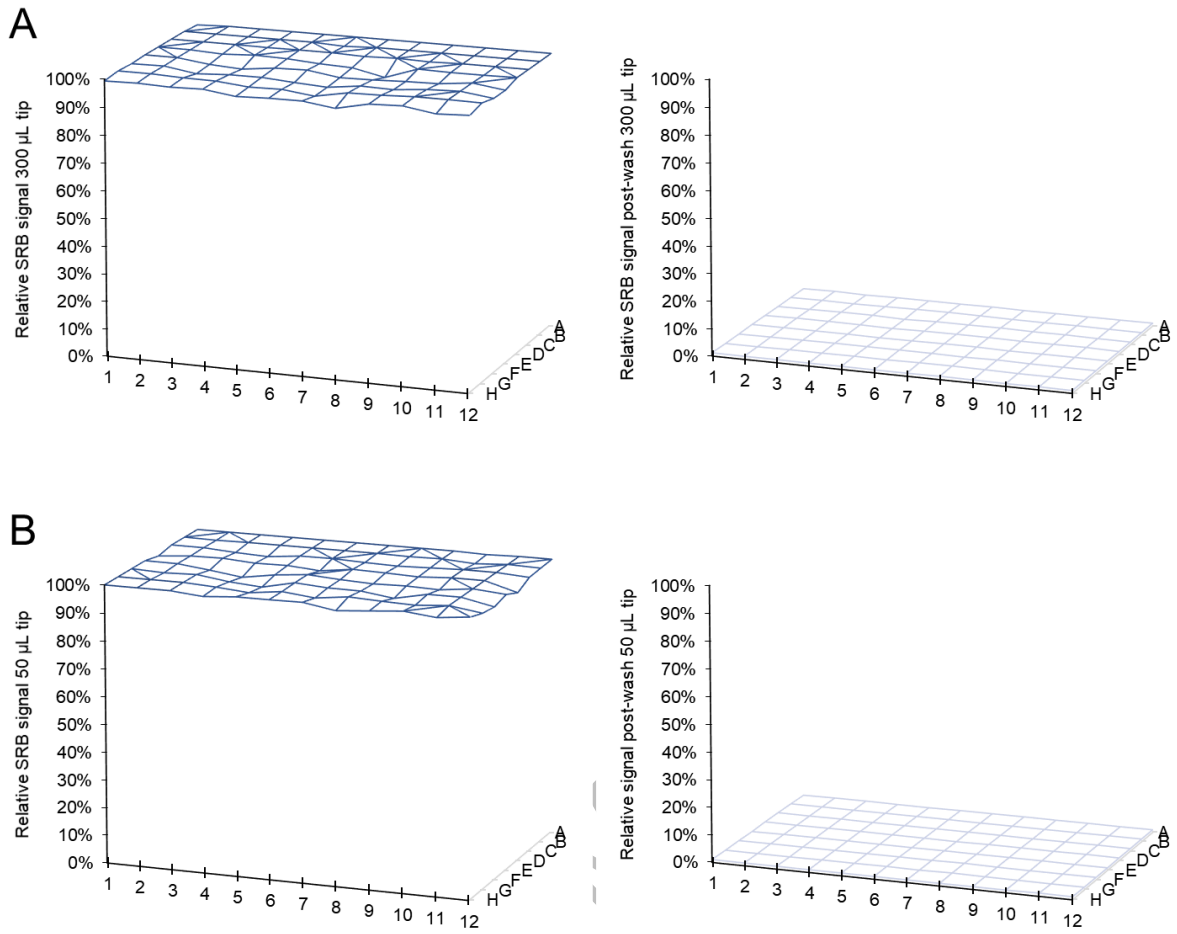

Figure 7. Tip wash routine demonstration with 100 µL SRB (0.004%) dispensed by standard 300 µL tip (A) or 50 µL tip (B) (n = 2).

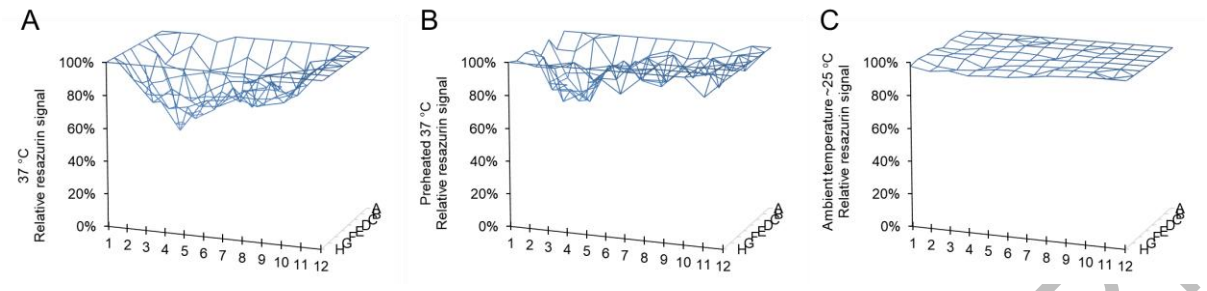

Figure 8. Edge effect demonstration of metabolic resazurin reduction assay (15 µM resazurin) with human fibroblasts incubated for 1 h with ambient temperature assay buffer in 37 ºC incubator (A), preheated assay buffer in 37 ºC incubator (B) or ambient temperature assay buffer and incubation (C).